

SUPPLEMENTARY METHODS

Yeast strain information¹⁸

EBY100 (**a** *GAL1-AGAL::URA3 ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS2 prb1Δ1.6R can1 GAL*), Trp⁻Leu⁻

EBY100 must be grown in rich media (YPD). Once transformed with the yeast display plasmid pCTCON2 (contains *trp1*), cells are grown in minimal media (SDCAA), and scFv expression is induced in galactose-minimal media (SGCAA). The pCTCON2 plasmid also carries ampicillin resistance for amplification in *E. coli*.

SDCAA plates

The following protocol is for 1 L or approximately 40-50 plates.

- 1** Mix 5.4 g Na₂HPO₄, 8.56 g NaH₂PO₄-H₂O, 182 g sorbitol, and 15 g agar in 900 mL ddH₂O. Autoclave.
- 2** Mix 20 g dextrose, 6.7 g Difco yeast nitrogen base, and 5 g bacto casamino acids in 100 mL ddH₂O. Filter sterilize.
- 3** Cool autoclaved mixture with stirring until below 50 °C. Add filter-sterilized components from Step 2.
- 4** Pour plates.

LB Amp plates

The following protocol is for 1 L or approximately 40-50 plates.

- 1** Mix 10 g tryptone, 5 g yeast extract, 10 g NaCl, and 15 g agar in 1 L ddH₂O. Autoclave.

- 2 Cool agar mixture with stirring until below 50 °C. Add filter-sterilized ampicillin solution to a final concentration of 100 µg/mL.
- 3 Pour plates.

Yeast library freezing

Reagents:

Low-dextrose SDCAA (5 g/L dextrose, 6.7 g/L Difco yeast nitrogen base, 5 g/L bacto casamino acids, 5.4 g/L Na₂HPO₄, 8.56 g/L NaH₂PO₄-H₂O)

Freezing solution (2% glycerol, 0.67% yeast nitrogen base)

Cryogenic vials

Isopropanol

- 1 Inoculate 10¹⁰ freshly grown cells into 1 L low-dextrose SDCAA and grow at 30 °C, 250 rpm for ~3 days.

Freezing cells in stationary phase aids their survival.

- 2 Pellet cells and remove supernatant.
- 3 Resuspend the cells in freezing solution to give a final cell concentration of ~6x10¹⁰/mL.
- 4 Aliquot at least 10¹⁰ cells per cryogenic vial. Incubate at room temperature for 10 min.
- 5 Place the vials in a bath of isopropanol at room temperature and slow-freeze the cells at -70 °C.
- 6 Transfer frozen yeast aliquots to liquid nitrogen for long-term storage.